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amino acids of RyR1 are predicted to fold into a second β-trefoil and an alpha helical domain. In this report, the relative orientation of these three domains (termed here as β1, β2 and α1) within the full-length RyR1 protein has been investigated using a novel FRET-based technique. This method monitors the relative proximity of a GFP fluorescent donor fused into RyR1 and a fluorescent acceptor, Cy3NTA, targeted to poly-histidine (His) tags inserted into the primary sequence of RyR1. In this study, GFP was fused to single positions within β1, β2 and α1 and FRET was then measured to Cy3NTA targeted to 6 His tags singly introduced into these three domains. The results of these measurements provide a detailed picture of the relative orientation of these three sub-domains within full-length RyR1 expressed in a cellular context. Further structural studies using this technique can now be undertaken to determine how these domains move during channel gating. (Supported by NIH grant R21ARO56406).

1030-Plat
3D Mapping of the SPRY2 Domain of RyR1 by Antibody Labeling and Single-Particle cryo-EM
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The ryanodine receptor (RyR1) and the dihydropyridine receptor (DHPR) interact functionally at the T tubule/sarcoplasmic reticulum interface of skeletal muscle and are main participants of the excitation-contraction coupling process. The SPRY2 domain of RyR1 has been shown to be relevant for the RyR1/ DHPR interaction. Here, using a combination of immunolabeling and single-particle cryo-EM we have mapped SPRY2 in the 3D structure of RyR1. RyR1 was incubated with three different antibodies against the SPRY2 domain and vitrified for cryo-EM imaging. The two main obstacles for the image processing procedure; limited amount of data and signal dilution introduced by the several possible binding locations of the antibody to the tetrameric RyR1, were overcome by modification of the 3D reconstruction scheme. This enabled us to obtain a 3D reconstruction of RyR1 with the antibody bound, which in turn has allowed us to map the SPRY2 domain in a T-tubule facing domain of RyR1.

1031-Plat
Localization of the Dantrolene Target Sequence in the Cardiac Ryanodine Receptor by Combining cryo-EM and FRET Analysis
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Mutations in ryanodine receptors (RyRs) cause calcium channel dysfunction in two skeletal muscle diseases, malignant hyperthermia (MH) and central core disease, and in polymorphic ventricular tachycardia in cardiac muscle. MH is an inherited condition that causes muscle rigidity and uncontrollable fever in reaction to administration of certain anesthetics. Dantrolene is a clinical drug that suppresses spontaneous Ca^{2+} release and is used as a therapeutic agent for individuals susceptible to MH. Recently, dantrolene has been shown to improve cardiomyocyte function in failing hearts. Biochemical studies suggest that the N-terminal region of RyR (amino acids 590-609 in RyR1 and 601-620 in RyR2) is the molecular target for dantrolene. We have attempted to localize the dantrolene target sequence by 3D cryo-electron microscopy. However, inserting a GFP after Arg-626 in RyR2 abolished the receptors' binding to the GST-FKBP12.6 affinity column and prevented the purification of $RyR2_{R626\text{-}GFP}$ for structural studies. As an alternative approach, we designed several fluorescence resonance energy transfer (FRET) pairs based on our previous cryo-EM structures to map the location of Arg-626. We generated four pairs of dual insertions in RyR2 ($RyR2_{S437\text{-}YFP/R626\text{-}CFP}$, $RyR2_{R626\text{-}CFP/Y846\text{-}YFP}$, $RyR2_{S437\text{-}YFP/Y846\text{-}CFP}$, and $RyR2_{R626\text{-}YFP/S2367\text{-}CFP}$); three pairs between AF555-FKBP12.6 and RyR2-GFP ($RyR2_{S437\text{-}GFP}$, $RyR2_{R626\text{-}GFP}$, and $RyR2_{Y846\text{-}GFP}$); and three pairs between CFP-FKBP12.6 and RyR2-YFP ($RyR2_{S437\text{-}YFP}$, $RyR2_{R626\text{-}YFP}$, and $RyR2_{Y846\text{-}YFP}$). We estimated the 3D location of dantrolene binding sequence by measuring the FRET efficiencies in each of these pairs, and by correlating FRET efficiencies to the distance between donor and acceptor with known locations. Our results reveal the dantrolene target sequence is likely to be located in the clamp region close to the FKBP12.6 binding site.

1032-Plat
Studies in the Binding of Calmodulin to Skeletal and Cardiac Ryanodine Receptors
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Ryanodine receptors (RyR) are calcium release channels that are modulated by cytosolic Ca^{2+}. They are a critical part in the process that leads to the contraction of skeletal and cardiac muscle. Calmodulin (CaM) is a ubiquitous calcium sensor that can fine-tune the Ca^{2+} sensitivity of RyR by binding to regions exposed to the cytoplasm. This modulation is isoform specific, depending on the amount of free cytosolic Ca^{2+}. Here we used isothermal titation calorimetry to determine the thermodynamic parameters of CaM binding to three distinct regions in cardiac (RyR2) and skeletal muscle (RyR1) channels. Coupled with alanine scanning, we have identified the critical anchor points in both regions which mediate CaM binding to RyR. We show that CaM binding to RyR is isoform- and lobe- specific at high Ca^{2+} concentrations. At low Ca^{2+} concentrations however, both isoforms behave similarily. The differences may underlie the different functional effects of CaM on RyR1 and RyR2.

1033-Plat
The N-Terminal Disease Hot Spot of Ryanodine Receptors Forms a Cytoplasmic Vestibule
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Ryanodine receptors (RyR) are ion channels that govern the release of Ca2+ from the endoplasmic reticulum. They thus regulate the contraction of skeletal and cardiac muscle. Mutations in RyR can lead to severe genetic conditions, including (but not limited to) malignant hyperthermia (MH) and catecholaminergic polymorphic ventricular tachycardia (CPVT). Despite the detailed investigation of the functional effects of the mutations, locating their position in the full-length channel structure has traditionally proven to be difficult. Here we present the 2.5 Ångström resolution crystal structure of a region spanning most of the N-terminal disease hot spot (residues 1-559), containing over 50 disease mutations in RyR1 and RyR2. The hot spot consists of three domains that interact through a predominantly hydrophilic interface. We have been able to dock the position of this hot spot into various RyR1 cryo electron microscopy maps, allowing its unambiguous positioning in the cytoplasmic portion of the channel, forming a 240-kDa ring around the fourfold symmetry axis. The disease mutations can be grouped into three different categories, either destabilizing the interfaces between the three N-terminal domains, affecting the folding of individual domains, or affecting one of six interfaces with other RyR parts. We propose a model whereby the opening of RyR coincides with allosterically coupled motions within the N-terminal domains. This can be affected by mutations that target various interfaces within and across subunits. We also propose a mechanism whereby RyRs are activated by redox modification through the destabilization of observed domain-domain interfaces. The structure provides a framework to understand the many disease mutations that have been studied using functional methods.

SYMPOSIUM 11: Interfaces Between Cells and the Outside World: Bioengineering Meets Biophysics

1034-Symp
Vertical Nanopillars For Highly-Localized Fluorescence Imaging in Live Cells
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The rapidly evolving field of nanotechnology creates new frontiers for biological sciences such as quantum dots for fluorescence imaging and nanotransistor-based biosensors. Recently, vertical nanopillars protruding from a flat surface has been shown to support cell survival and deliver large molecules into the attached cells. Here we demonstrate (1) the use of vertically aligned SiO2 nanopillars to achieve below-the-diffraction limit observation volume in vitro and inside live cells and (2) the use of vertical Pt nanopillars to enhance electrical coupling between neuron cells and the recording electrodes. Transparent SiO2 nanopillars embedded in a nontransparent substrate restricts the propagation of light and affords evanescence wave excitation along its vertical surface. This effect creates high-localized illumination that can be used for single molecule detection with high fluorescence background. We also fabricate vertically aligned Pt nanopillars to enhance the electrical coupling between cultured